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Topical Review

Tight Junctions and Apical/Basolateral Polarity

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Introduction

For a While, Epithelial Asymmetry Appeared in Violation of the Laws of Physics

The observation that a frog skin mounted between two identical salt solutions exhibits an electrical potential difference between its two sides (Fig. 1*a*) prompted Galeotti [40, 41], at the beginning of this century, to suggest that this preparation has a higher Na^+ permeability in the pond-to-blood direction than in the reverse one. However, biologists were reluctant to accept this hypothesis, because it appeared in violation of the laws of thermodynamics (Fig. 1*b*). Half a century later, measurements with radioactive tracers demonstrated that Galeotti was right: the influx of Na^+ is in fact much larger than its outflux, even in the absence of an electrochemical potential gradient. To account for this functional asymmetry Koefoed-Johnson and Ussing [59] proposed a model (Fig. 1*c*) based on the polarized distribution of ion-translocating mechanisms in the membrane of epithelial cells: passive Na-permeating mechanisms were assumed to be located on the apical, and Na-K pumps on the basolateral side. The information accumulated thereafter confirmed these assumptions and indicated that membrane polarity is not restricted to the distribution of ion-translocating mechanisms, but is a general characteristic of membrane molecules of epithelial cells.

Since the model depicted in Fig. 1*c* was developed with information obtained almost exclusively with the frog skin (a preparation with a high transepithelial electrical resistance), the possibility that fluxes occurring through the intercellular pathway may play a significant role in other epithelia was momentarily neglected. In fact, the information that this pathway is blocked by an "occluding bar" was available since the beginning of this century [9, 164]. This was confirmed by the work of Farquhar and Palade [35] who demonstrated that this "bar" is constituted by a junctional complex in which the tight junction (TJ) acts as a sealing element preventing the diffusion of extracellular markers. However, intense research started in the 60's offered clear evidence that most of the flux of ion and water across epithelia, such as the gallbladder, the intestinal mucosa, the choroid plexus and the proximal tube of the kidney, takes place through the paracellular pathway controlled by the TJ (Fig. 1*d*), indicating that this structure is by no means an "occluding bar," but functions instead as a selective barrier [10, 11, 24, 31–33, 36, 49, 56, 93, 125, 159–161].

Therefore, in the early 70's apical/basolateral polarity and TJs were recognized as the two differentiated features that enable epithelial membranes to act as permeation barriers and carry out the exchange of substances between higher organisms and the environment. A series of concepts prevailing in those days led to the assumption that polarity depends on TJs: (i) the concept that membrane proteins were free to diffuse in the plane of the membrane [37] and that a fence was needed to prevent those in one pole of the cell to migrate to the wrong side; (ii) the assumption that the randomization of previously polarized membrane components produced by Ca-chelating substances like EDTA and EGTA is due to the opening the TJ [42, 51, 140]; and (iii) the assumption that a barrier like the TJ would be able *to sort* apical and basolateral membrane proteins. The observations on which these assumptions were based have been subsequently reinterpreted, and the purpose of this review is to assess the relationship between TJs and apical/basolateral polarity, starting with a brief description of these two features of epithelial cells.

Key Words tight junctions · cell polarity · epithelial · apical · basolateral · vectorial

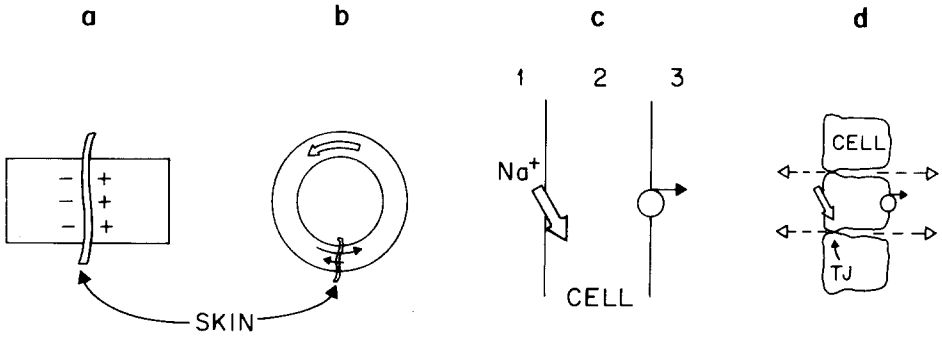

Fig. 1. Evolution of the ideas on apical/basolateral polarity and tight junctions. (*a*) At the beginning of this century, Galeotti proposed that the electrical potential difference across a frog skin mounted between two identical saline solutions is due to a higher Na-permeability in the pond-to-blood than in the opposite direction. (*b*) There was a concern that, due to this asymmetric permeability, a frog skin mounted in a doughnut-shaped chamber would decrease the Na^+ concentration on the outer side (*left*) and increase it on the inner side; the gradient thus created would produce a counter-clockwise diffusion of Na^+ (open arrow), and create a *perpetuum mobile*. In fact, the system does not last perpetually, because the skin exhausts its metabolic resources and the asymmetry vanishes. (*c*) The use of Na tracers demonstrated that Galeotti's assumption is correct, and Koefoed-Johnson and Ussing (KJU) proposed an asymmetric model for the epithelium (compartment *2*), in which Na^+ passively penetrates into the cell from the outer solution (compartment *1*) and is actively pumped towards the inner bathing one (compartment *3*). (*d*) Adaptation of KJU's model to "leaky" epithelia required a special consideration of the paracellular route (dashed arrows) limited by the TJ

What is a Tight Junction?

Tight junctions circle the cell at the limit between the apical and the lateral regions. They appear in thin transverse sections as a series of punctuate contacts between the cell membrane of neighboring cells, that block the diffusion of extracellular markers for electron microscopy such as ferritin, horseradish peroxidase, lanthanum and ruthenium red [75, 123, 157]. On glutaraldehide fixed specimens TJs appear in P faces of freeze-fracture replicas as a pattern of branching and anastomosing ridges, and in E faces as furrows in register with the ridges on P faces [14, 25, 50, 82, 143, 146, 155]. In unfixed material the location of ridges and furrows is reversed. Current models consider that these ridges (also called strands) are constituted by chains of proteins [21, 28, 145, 147] in one cell membrane, that close the intercellular gap by binding to those of the neighboring cell. An alternative view favors the possibility that strands consist of cylindrical lipidic micelles that circle the cells and have their polar head groups oriented towards the hydrophilic core and their hydrophobic chains pointing outwards and fusing with the matrix of the membranes [55, 86, 114]. Recent reviews deal with the synthesis, assembling and sealing of TJs, relationship between their structure and their degree of sealing, association with the cytoskeleton, relationship to other intercellular contacts and cell-attaching molecules, and with their variations in response to drugs and physiological conditions [17–20, 45, 137, 144].

Apical/Basolateral Polarity

As mentioned above, the concept of apical/basolateral asymmetry arose as a necessity to explain vectorial transport of substances across epithelial membranes (Fig. 1). This concept was since substantiated by electron microscopic studies, cell fractionation of apical and basolateral membranes, differential attachment of fluorescent and radioactive probes and other experimental procedures mentioned below, and its present status is detailed below.

Anatomical Features

Apical and basolateral domains usually differ in the size of their surface areas [80, 142, 152, 156], the presence of microvilli, desmosomes and gap junctions and in the number of intramembrane particles per unit area [18, 42, 44, 51, 85].

Proteins

Different Protein Species May Exhibit Opposite Polarity in the Same Cell

Thus, while Na-K-ATPase [61, 68, 84, 90, 141], adenylate cyclase [124, 139], hormonal and immunoglobulin receptors [110, 111, 149], sugar [58, 120] and aminoacid transport systems [1, 43, 121], desmosomal and gap junctional proteins may be

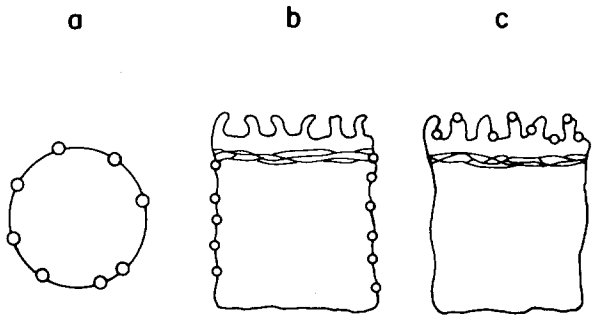

Fig. 2. A given membrane protein (in this example Na-K-ATPase represented by circles) shows no polarity in an erythrocyte (*a*), a basolateral position in an epithelial cell from the gallbladder (*b*), and an apical location in a cell from the choroid plexus (*c*)

found on the basolateral side of an epithelial cell, others like leucine aminopeptidase [30, 57, 68, 131, 132], the amiloride-sensitive sodium channel [66, 106], dipeptidyl peptidase [52], α-glutamyl transferase [54, 74] and the Na-glucose [95, 119] and Na-amino acid cotransporters [97, 98, 122, 158] are found in the apical region.

A Given Membrane Protein May Be Polarized in Some Cell Types but Not in Others

Thus Na-K-ATPase exhibits an approximately homogeneous distribution in the cell membrane of an erythrocyte (Fig. 2*a*), but occupies only the basolateral side of the gallbladder, hepatocyte, pancreas, urinary bladder, frog skin and kidney tubular cells (Fig. 2*b*) [8, 15, 61, 84, 89, 90, 141].

A Given Protein May Show Opposite Polarity in Two Different Epithelial Cell Types

Thus Na-K-ATPase is found in the basolateral region of cells of most epithelia (Fig. 2*b*), but is placed in the apical domain of those of the choroid plexus [118] and the retinal pigmentary epithelium (Fig. 2*c*). This biochemical asymmetry allows epithelial cells to pump ions vectorially. A physiological alternative to this biochemical asymmetry is provided by an "enzymatic breakage" elicited by the lipidic matrix [64]. As detailed below, lipids in the apical pole of epithelial cells have a tighter packing than the basolateral ones, and this may reduce the activity of certain enzyme and translocators [65]. Conversely, a fluidification produced either by changes in the lipid composition or by agents such as A_2C, benzyl alcohol and Triton enhances enzyme activity [60, 92]. Le Grimellec et al. [63] and Sutherland et al. [148] have recently shown that dormant Na-K pumps on the apical domain may be activated in this way.

A Given Protein May Be Located at Different Poles of the Same Cell Type

Thus Brown et al. [13] have found that some intercalated epithelial cells of the cortical collecting duct of the rat kidney have their H^+-ATPases inserted in the apical pole, while neighboring cells of the same type exhibit just the opposite polarity.

Different Proteins in the Same Cell May Differ in their Degree of Polarity

The degree of apical/basolateral polarization of a given protein species is usually assessed through binding of fluorescent, radioactive and chemical probes to the plasma membrane or to fractions originated from different membrane domains [38, 67, 84, 117]. These techniques are not devoid of errors due to contaminations with other fractions, nonspecific binding and background signals. In spite of these sources of uncertainty, different membrane protein of the same cell may exhibit a wide range of apical/basolateral polarization, so that for a given species this ratio may be 100% while in another protein species it may only be 10% [128].

In summary, the "logic of the system" seems to be: free living cells have an approximately even distribution of pumps, channels carriers, receptors; whereas in polarized cells, especially in epithelial cells, all these elements are redistributed to subserve an organismic function in addition to the cell's own needs. This "logic" is clear in most cases. The cells may control either actual protein distribution or protein activity (via lipid "pressure" packing) to achieve the same results.

Lipids and Glycolipids

Although lipid molecules are free to diffuse in the plane of the membrane, their populations in the apical and the basolateral membrane are not identical [81, 102, 103, 150, 151] nor have the same degree of fluidity [62, 63, 65, 148]. This difference is mainly due to the composition of the outer leaflet. The TJ blocks the diffusion towards the basolateral region of lipid probes introduced in this leaflet (Fig. 3, upper) [34, 143, 151] except for those probes that have the ability to flip-flop to the inner leaflet (Fig. 3, lower) [34, 143], or that are fused directly to this layer [151].

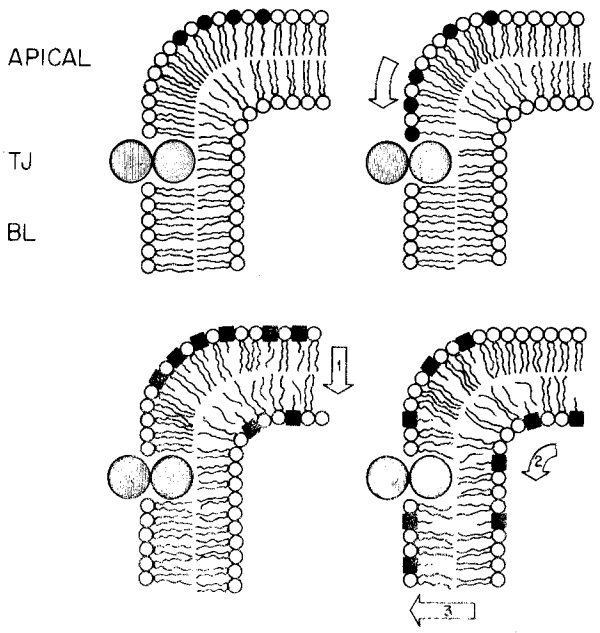

Fig. 3. A lipid probe (black heads) inserted in the outer leaflet of the apical membrane (upper left), is prevented from diffusion to the basolateral side by the presence of the TJ (upper right). However, if the lipid probe has the ability to flip-flop toward the inner leaflet (lower left, arrow *1*) it may diffuse toward the basolateral region (lower right, arrow *2*) and flip-flop back to the outer leaflet (arrow *3*)

The Origins of Apical/Basolateral Polarity

A number of cellular mechanisms were found to be involved in the achievement of asymmetry between the apical and the basolateral membranes.

Molecular Sorting. Rodriguez-Boulan and coworkers have shown that polarized budding of influenza virus (through the apical) and stomatitis virus (through the basolateral) depends on vectorial insertion of their envelope proteins HA and G, respectively [77, 91, 115, 126, 128–130, 136]. Likewise, Caplan et al. [16] have found that once synthesized, the α-subunit of the Na-K-ATPase is delivered only to the basolateral membrane of MDCK cells.

Membrane Recycling. The cell membrane undergoes a continuous retrieval and restoration process that, nevertheless, does not randomize its components [112]. In the case of epithelial cells, this implies that the cell correctly sorts and addresses the membrane vesicles that should be fused to the apical or to the basolateral regions. Thus leucine aminopeptidase is removed from the apical domain of MDCK cells and reinserted a few minutes later in the same domain [68].

Default Polarization. At least in principle, a membrane protein may accumulate in one pole of the cell as a result of lacking the necessary signal to be addressed to the opposite side. Likewise, a protein may reach the surface membrane due to a lack of the signal needed to be retained in intracellular compartments. This seems to be the case of some proteins that normally belong to intracellular organelles and that are known to reach the surface membrane or have even been secreted when they lack the tetrapeptide Lys-Asp-Glu-Leu or other fragments of their chains [70, 78, 99, 107, 116].

Mixing of Signals. There is a number of proteins and fractions of proteins whose insertion in the plasma membrane may be readdressed when their molecules are supplemented with fractions of molecules which normally have the opposite vectorial insertion [83, 133].

Anchoring to the Cytoskeleton. The attachment of proteins to the underlaying cytoskeleton helps to retain them in an asymmetric position [*see,* for instance, 105). Thus Nelson and Veshnock have found that fodrin (an analog of spectrin) forms an insoluble network in contact with the basolateral region of MDCK cells, and Na-K-ATPase is retained in this region due to its binding to fodrin [100, 101]. On the contrary, when proteins are free to drift in the plane of the membrane and reach the wrong pole of the cell, they can be easily extracted from this position with Triton X-100 [135]. However, the asymmetry of other proteins does not appear to depend directly on attachments to the cytoskeleton. Thus disruption of microtubules and microfilaments with drugs like colchicine, nocodazole and cytochalasin was observed to result in missorting or reduction of the degree of polarization of HA proteins of influenza virus, but do not interfere with the delivery of G protein from stomatitis virus to the basolateral surface [127, 134].

Interaction with the Extracellular Matrix or with Neighboring Cells. Apical—but not basolateral—markers may show a certain degree of polarization in cells whose normal attachment to a substrate or to another cell is impaired [3, 29, 152]. Blockade of cell attaching molecules (CAMs) with antibodies prevents the polarization of membrane proteins in MDCK cells as well as the assembly of elements of the junctional complex which are normally placed on the lateral side [47, 53].

Anchorage to Glycosyl-Phosphatidylinositol (PI). There is a class of glycoproteins whose extracellular domain is a polypeptide linked to a phos-

phatidic molecule of the membrane via an ethanolamine, a glycan and an inositol [69]: alkaline phosphatase, renal dipeptidase, trehalase and 5'nucleotidase. Noticing that these are typical markers of the apical region, Lisanti et al. [67] suspected that their asymmetry is due to this special anchorage and devised an assay to cleave the peptide moiety with a phosphatidylinositol-specific phospholipase C from the apical or from the basolateral side. In this way they identified six new proteins linked to phosphatidic molecules and found that all of them were restricted in fact to the apical side.

Degree of Differentiation. In some cells the polarized distribution of enzymes and co-transporters is strongly dependent on cell density and differentiation-inducing chemicals [2, 96, 119, 162, 163].

The Fence Constituted by the TJ Contributes to Polarity in Two Ways. First, as mentioned above, some molecules, in particular lipids which do not have an appreciable flip flopping rate (Fig. 3) and proteins which exhibit free diffusion coefficients in the plane of the membrane, may be restricted to the apical or to the basolateral regions by the fence interposed by the TJ. However, while this mechanism may help to *maintain* a polarized distribution, it may not be responsible for the *achievement* of polarity. Second, by maintaining the asymmetry of lipidic composition and in particular the tightness of their packing, the TJ helps them to subdue the activity of enzymes and carriers of a given region [63].

Polarized Proteins May in Turn Modify the TJ. Thus the activation of Na-coupled transport of glucose, alanine or leucine in the intestinal mucosa elicits a decrease of the electrical resistance of the TJ [108, 109] accompanied by the development of large dilatations between its strands [73]. This effect seems to be mediated by a contraction of the perijunctional actomyosin ring in the vicinity of the TJ [72, 73].

Polarity May Be Achieved in the Absence or Even in Spite of Tight Junctions

Figure 4 illustrates several situations in which the process of polarization appears to proceed regardless of the presence of the fence constituted by TJs: (i) MDCK plated at confluence and left for 20 hr in media with low Ca^{2+} (1–4 μM) do not form TJs [44] and do not have their Na-K-ATPase asymmetrically located in their basolateral membrane, but distributed over their entire surface membrane [29] (Fig. 4*a*). Upon addition of Ca^{2+} the TJ starts to form

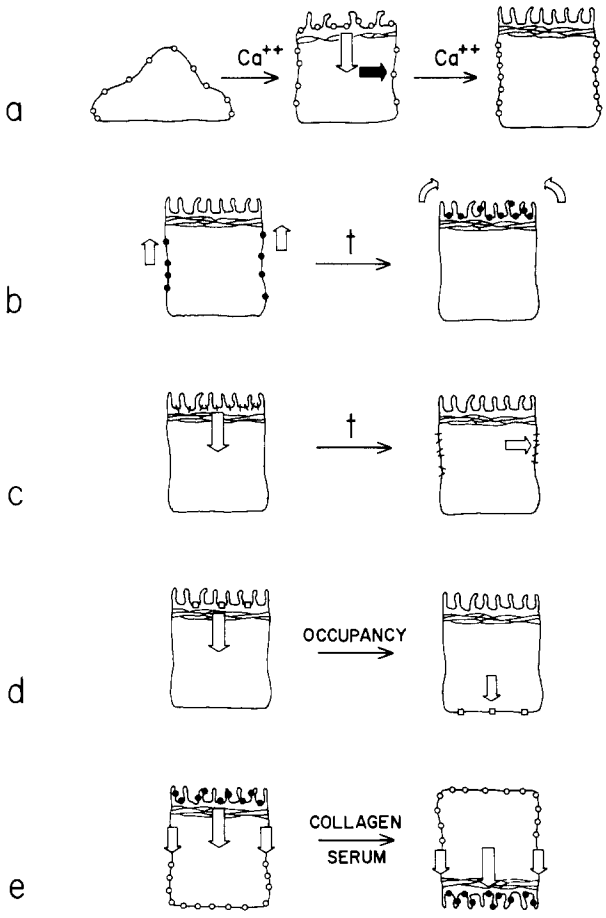

Fig. 4. Membrane proteins may achieve an apical/basolateral polarity in spite of the TJ: (*a*) Na-K-ATPase (circles) is not polarized in an MDCK cultured in Ca-free medium. Ca^{2+} addition triggers the formation of TJ (Fig. 5) and traps some Na-K-ATPases on the apical (wrong) side. The cell then removes apical Na-K-ATPases (open arrow) and inserts new enzyme in the basolateral membrane (*arrow*) until its typical polarity is achieved. (*b*) An epithelial cell from the intestinal mucosa first exhibits aminopeptidase N in its basolateral (wrong) membrane, but gradually displaces this enzyme toward the apical pole. (*c*) When G protein of VSV virus is fused to the apical (wrong) membrane using liposomes, the cell removes it from this location and reinserts it in the basolateral membrane. (*d*) Receptors occupied by IgG are removed from the basolateral position and transferred to the opposite pole of the cell. (*e*) In a thyroid cell of a follicle suspended in medium without collagen or serum, the apical side faces the medium. Upon addition of collagen or serum the cell reverses its polarity and relocates its TJ

(Fig. 5), thus trapping a fraction of the Na-K-ATPase on the apical (wrong) side. However, the enzyme is removed from this position and reaches its normal polarization in a few hours [29]. (ii) Fig. 4*b* depicts the situation that seems to prevail with apical markers in the intestinal mucosa as well as in hepatocytes. These markers are inserted first in the basolateral membrane, and migrate from this posi-

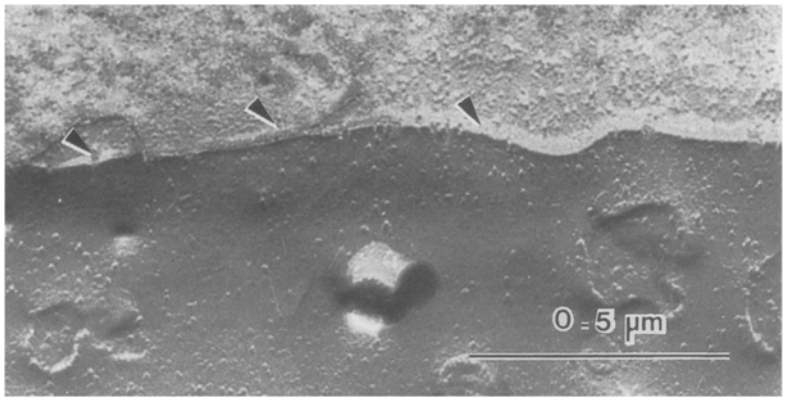

Fig. 5. Five to 15 minutes after the addition of Ca^{2+} to monolayers of MDCK cells that have been cultured in the absence of this ion, a junctional strand starts to form at the limit between the apical and the basolateral region, i.e., the position it will occupy in mature monolayers, as if its molecular components were delivered to the surface membrane in a polarized way

tion towards their permanent position in the apical membrane [6, 48, 76]. (iii) G protein (a protein that once synthesized in the cell is addressed to the basolateral surface) extracted from the envelope of stomatitis virus, incorporated into liposomes and fused into the apical membrane of MDCK cells, is quickly removed from this position and reinserted into the basolateral region [79, 113] (Fig. 4*c*). The difference between this process and the one described in Fig. 4*a* is that while G protein is removed from the apical and reinserted into the basolateral membrane, Na-K-ATPase is only removed from the apical, and the Na-K-ATPase inserted into the basolateral membrane is a new protein originated in intracellular compartments. (iv) Some membrane proteins are sorted by receptor-mediated endocytosis. The receptor-ligand complex is known to be trapped in coated pits, endocyted and transported to endosomes [39]. At the low pH of endosomes it dissociates, and ligands may be directed from the endosomes to the opposite surface of the epithelial cell (transcytosis). In some cases, the receptor itself is also transported from one pole to the other. A clear example of this process is the transport of immunoglobulins produced by plasma cells and transported across epithelia into a wide variety of secretions such as bile, milk, saliva, etc. [12, 94]. Another example of trancytosis but in the opposite direction takes place in suckling rats that acquire immunity by passage of milk IgG across the small intestine and into circulation (Fig. 4*d*). The fact that some 100 vesicles per minute traverse an MDCK cell in each direction gives an idea of the intensity of this process [142]. Such a transcellular flow of membrane necessarily implies that sorting must take place during transcytosis to avoid a rapid intermixing of apical and basolateral components. (v) Cells isolated from the thyroid gland and kept in suspension form follicles in which microvilli point towards the bathing medium and TJs occupy the outermost end of the intercellular space (Fig. 4*e*). However, exposure of this surface to collagen provokes the formation of a lumen at the core of the follicle, reversion of polarity, and migration of the TJ in the plane of the lateral membrane until it occupies the innermost end of the intercellular space [4, 5, 26, 27, 104]. HCO_3-secreting cells of the cortical collecting duct of the kidney possess Cl^-/HCO_3^- exchangers in the apical domain and proton pumps in the basolateral membrane. The polarity of these ion translocating mechanisms can be induced to reverse by acid-loading the animal [138]. An alternative explanation would be that, under this loading, a cell with a given polarity is switched off and a neighbor with the opposite polarity is switched on [13].

Do TJs Depend on Polarity?

The examples mentioned in the previous section clearly indicate that, contrary to early expectations, apical/basolateral polarity does not depend on TJs. Ironically, the information gained with the use of cultured epithelial cells that polarize and form TJs in vitro [18, 19, 22, 23] suggests that the reverse may not be true and that the making and positioning of a TJ may depend on some of the mechanisms of membrane polarization listed above. Thus, when Ca^{2+} is added to monolayers of MDCK cells kept for 20 hr in the absence of this ion, a single junctional strand appears in freeze fracture replicas in a few minutes (Fig. 5), already occupying the characteristic position exhibited by TJs in mature monolayers, and further strands develop in association with the initial one, as if molecular components previously contained in cytoplasmal compartments were added directly to the junctional belt by a sharply polarized mechanism [44]. Upon addition of Ca^{2+}, antibodies against ZO-1, a protein associated with the TJ [147], are transferred from inner com-

partments to the surface membrane at a region immediately adjacent to the still incomplete junctional bands [153]. The possibility that the positioning of the TJ were another manifestation of an overall polarizing mechanism, is further stressed by the fact that TJs maintain their positions through a close association with the cytoskeleton [7, 71, 87, 88] and is stabilized through Ca-dependent cell-cell contacts [46, 47, 154].

Summary

Physiological studies led to devise models of epithelial cells in which the membrane does not have its molecules distributed homogeneously, but polarized towards the apical or towards the basolateral regions. For a while, it was assumed that the TJ, acting as a fence between the two regions, would be responsible for this asymmetry. However, today the information available indicates not only that polarization may proceed independently of the TJ, but that this structure itself may attain its precise location due to a polarization process. Nevertheless, TJs may play a role in restricting to the apical or to the basolateral region those molecules that are free to diffuse in the plane of the cell membrane (e.g., lipids and protein that are not attached to cytoplasmic or extracellular structures).

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